Clinical Science

This test has been adapted for studies on serum antibodies accompanying skin homotransplantation in rabbits. Liver tissue of donor origin was treated with recipient's serum and used for absorption of Coombs' serum. The antibodies were true iso-antibodies and never acted upon the animal's own tissue.

Antiglobulin Consumption Test in Rabbit Homotransplantation

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THE FORMATION OF humoral antibodies in response to homotransplantation is a well-established phenomenon; both hemagglutinins and cytotoxins have been quantitated.[1] In previous work from this department, antibodies accompanying homograft rejection were studied by means of skin tests (local inverse anaphylaxis)[2-3] and mixed agglutination in tissue cultures.[4]

Although the relationship of humoral antibodies to graft destruction has not been established, evidence is accumulating which suggests that the role of these antibodies is quite important.[5-9] This increases the need for various serologic methods of studying these antibodies.

This paper describes the application of the antiglobulin consumption test to the study of humoral antibodies produced as a result of skin homografts in the rabbit.

Materials and Methods

Operative Procedure.—Adult rabbits of several varieties were used, some colored and some albino. Full-thickness skin grafts were transplanted in various sex and color combinations under local anesthesia.

Thirteen pairs of rabbits were studied. In ten pairs, each animal served as either donor or recipient; while in three pairs, each animal served as both donor and recipient. Grafts, oval in shape, were taken from the back and transplanted orthotopically. The dimensions of the first grafts were 15 × 7 cm in ten animals, 7 × 4 cm in four animals and 4 × 2 cm in two animals. All second and third grafts were 7 × 4 cm. Either Michel's clamps or silk sutures were used to hold the graft in place, and no dressing was applied. Skin from the original donor was also used for second and third grafts whenever they were performed. Following grafting, blood samples were taken from the ear artery at varying time intervals and the serums were stored at —20 C. Ultimately, both donor and recipient were killed by cardiac bleeding. Their organs were harvested and stored at —20 C.

Preparation of Antigens.—A suitable piece was cut from the frozen liver without thawing the organ. This piece was then thawed and made into a 30% sodium chloride suspension by blending it in a high-speed blender for 15 minutes. The suspension was spread in a thin film onto a pane of glass and allowed to dry at room temperature. The dry tissue resulting was scraped from the glass and pulverized by forcing it through a fine wire screen.

Agglutinating Antiglobulin System.—Procedures outlined in a previous publication [10] were followed. Anti-G serum was obtained by immunization of blood group gg rabbits with rabbit G erythrocytes.[11] The anti-G serum used in this study had a hemagglutinating titer of 1:8 and incomplete antibody titer of 1:1000.

Erythrocytes from blood group G rabbits were washed three times with phosphate-buffered sodium chloride solution (pH 7.2) and prepared as a 2% suspension. The erythrocyte suspension was mixed with an equal volume of anti-G serum at 1:30 dilution and incubated for 30 minutes at 37 C. The sensitized erythrocytes were washed three times and resuspended to a 1% concentration.

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